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Issued September 5, 1911.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF SOILS—BULLETIN NO. 80.
MILTON WHITNEY, Chief.

EXAMINATION OF SOILS FOR ORGANIC CONSTITUENTS, ESPECIALLY DIHYDROXYSTEARIC ACID.

BY

OSWALD SCHREINER,
Scientist in Charge of Fertility Investigations,
AND
ELBERT C. LATHROP,
Scientist in Fertility Investigations.

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BUREAU OF SOILS.

MILTON WHITNEY, *Chief of Bureau.*

ALBERT G. RICE, *Chief Clerk.*

SCIENTIFIC STAFF.

FRANK K. CAMERON, in charge of Physical and Chemical Investigations.
CURTIS F. MARBUT, in charge of Soil Survey.
OSWALD SCHREINER, in charge of Fertility Investigations.
W J McGEE, in charge of Soil Water Investigations.

SCIENTISTS IN FERTILITY INVESTIGATIONS.

Edmund C. Shorey.
M. X. Sullivan.
B. E. Brown.
J. J. Skinner.
F. R. Reid.
E. C. Lathrop.
J. H. Beattie.
A. M. Jackson.
H. Winckelmann.

LETTER OF TRANSMITTAL.

. S. Department of Agriculture,
Bureau of Soils,
Washington, D. C., April 15, 1911.

Sir: I have the honor to transmit herewith the manuscript of a scientific paper entitled "Examination of Soils for Organic Constituents, especially Dihydroxystearic Acid," by Dr. Oswald Schreiner and Mr. Elbert C. Lathrop, of this bureau. This article embodies the results of an investigation into the nature of the organic matter of many soils from widely separated localities. The importance of the organic matter in soils makes these results especially interesting, and the article throws much light upon the question of soil fertility. I have, therefore, the honor to recommend that this article be published as Bulletin No. 80 of the Bureau of Soils.

Respectfully,

Milton Whitney,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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EXAMINATION OF SOILS FOR ORGANIC CONSTITUENTS, ESPECIALLY DIHYDROXYSTEARIC ACID.

INTRODUCTION.

That infertility or unproductivity in soils is not always determined by a lack of beneficial elements like nitrogen, phosphorus, or potassium, as revealed by chemical analyses, has been repeatedly emphasized in former publications from this bureau and is being recognized more and more, both in this country and abroad, judging from the published reports of investigations during the last three or four years. Not all of these investigators attribute this failure of chemical analysis to account for the facts to the same cause, nor is it to be expected that any one cause is entirely responsible for such a condition, but rather that different causes contribute to the same result in different soils. In the present bulletin it is the purpose to emphasize, and emphasize rather strongly, one of the reasons why soils may be infertile and yet have a sufficient, if not plentiful, supply of the usual plant nutrients. The fact here to be emphasized, therefore, is that soils may be unproductive, not because a beneficial substance or substances are absent, but because a substance or substances deleterious to the growing crops are present. This idea, really a very old one, has met in past times with much favor and much criticism. It has been adopted by agricultural philosophers and discarded by agricultural investigators, adopted by the former because the idea seemed logical and right and explained many facts, but discarded by the latter because no adequate proof could be furnished by science in its undeveloped state at that time.

By the application of modern methods of research to the intricate problems of the soil, this laboratory has proved conclusively that harmful substances, whatever may be their origin, do exist in certain soils. And furthermore, through the application and extension of the biochemical advances made in other fields of scientific endeavor, it has been possible to throw such light upon the biological and biochemical changes in soils that the old views of soil organic matter, soil humus, and the process of humification are entirely overthrown.

The first intimation of the presence of harmful soil constituents in some soils was obtained through the behavior of plants in water extracts from such soils. This can be shown very readily by anyone with such a soil under investigation. The soil is shaken with distilled water for several minutes, allowed to settle, and the supernatant liquid filtered off, which must usually be done with a Pasteur-Chamberland filter in an apparatus specially designed for such work, in order that all solid material may be removed. The clear filtrate is then used as a medium for the growth of wheat seedlings in bottle cultures. A control in pure distilled water, or in an extract from a fertile soil, should be run at the same time. In such cases the plants grown in the extract from the poor soil will show many peculiarities not shown by the plants either in pure distilled water or in the extract from the good soil. The plants will be smaller, less developed in top and root, the latter often showing dark and swollen tips, which are sometimes bent into hooks, a phenomenon characteristic of certain toxic action. The growth in this soil extract may even be greatly less than the growth in the distilled water, although the soil extract naturally contains plant nutrients, whereas the distilled water contains none.

If a separate portion of the original extract be treated with carbon black, made from natural gas by imperfect combustion much as lampblack is formed on lamp chimneys, agitated and filtered, the filtrate will be a good medium for the growth of the seedlings. This simple treatment with carbon black has, therefore, removed by absorption the harmful properties of the soil extract and the growth is now even better than in the distilled water.

Observations of this kind on many soils, together with a study of the properties of the material dissolved in the water, led to no definite isolation of the compound or compounds showing the harmful effect, owing to the fact that the quantities in the water extract are too small for identification, but they did lead to a recognition that the substances were not mineral in character, but were constituents of the organic matter of the soils.

That organic substances could produce such effects in such small quantities as must be present in soil solutions was not apparent from the literature and it became necessary to establish this point. With this in view, a test of about 40 substances of organic origin which may get into the soil or be formed therein were tested, and in Bulletin 47 it was conclusively shown that a number of these were decidedly harmful to plants, even in very dilute solutions, comparable with the organic content of soil solutions.

It therefore became essential to make a study of the organic matter of the soil. The organic matter of the soil, however, was a subject about which the older chemistry of agriculture had much to say, but in regard to which modern science was discreetly silent. With no established facts and no methods of attack worked out, progress was necessarily slow at first, but gained speed with each compound isolated or identified until to-day there have been isolated in these laboratories more than 25 definite compounds from soil organic matter and the work is progressing at a rapid pace.

The isolation of harmful constituents is naturally only a phase of the entire field covered by these investigations. Some of the compounds are not only not harmful but even beneficial, as will be brought out in subsequent reports.

Having found a compound in one or two soils and devised a method for its isolation and identification, the question naturally arises: Is this compound peculiar to the soil from which it has been isolated or is it a general soil constituent? Does it appear in one and the same soil under all conditions or is it the result of special treatments or crop practices? It is obviously of the greatest importance to know under what soil conditions and in what soils or class of soils any particular compound or group of compounds exists, in order that a true and comprehensive view of soil organic matter in these soils can be obtained.

Among the harmful compounds is one which has been rather thoroughly studied, both in respect to its chemical properties and its action on plants. This compound is dihydroxystearic acid. Moreover, its method of isolation, while rather difficult, is simple in comparison to the methods necessary for the isolation or identification of other soil compounds. For this reason a special examination of soils from widely different areas in the United States, as well as soils formed and existing under widely different conditions, was made and the reports of this examination, as well as a report on the distribution of other soil constituents, is given in this bulletin.

ORGANIC CONSTITUENTS ISOLATED FROM SOILS.

There have been isolated from soil organic matter since this work was begun in these laboratories a large number of compounds[1] which are so varied in their composition, properties, and origin that they may be said to cover fairly well the principal classes of compounds encountered in other lines of biochemistry, and, therefore, the knowledge in regard to the chemical relationship, origin, and processes of change accumulated in such other lines can be directly applied to the understanding of the biochemical changes in soils and the constitution of soil organic matter.

Of the compounds thus far isolated, some contain only carbon and hydrogen; some carbon, hydrogen, and oxygen; some carbon, hydrogen, oxygen, and nitrogen; and some carbon, hydrogen, oxygen, nitrogen, and phosphorus. These compounds fall into the following classes: (1) Paraffin hydrocarbons, represented by *hentriacontane;* (2) hydroxy fatty acids, represented by *α-monohydroxystearic acid* and *dihydroxystearic acid;* (3) organic acids of unknown constitution, represented by *agroceric acid, paraffinic acid, lignoceric acid*, and a number of *resin acids;* (4) esters and alcohols, represented by *agrosterol, phytosterol, glycerides of fatty acids*, and *resin esters;* (5) carbohydrates, represented by *pentosans* and *pentose sugars;* (6) hexone bases, represented by *histidine* and *arginine;* (7) pyrimidine derivatives, represented by *cytosine;* (8) purine bases, represented by *xanthine* and *hypoxanthine;* (9) pyridine derivatives, represented by *picoline carboxylic acid.*

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[1] The Isolation of Harmful Organic Substances from Soils, by Oswald Schreiner and Edmund C. Shorey. Bul. 53, Bureau of Soils, U. S. Dept. Agr. (1909). Chemical Nature of Soil Organic Matter, by Oswald Schreiner and Edmund C. Shorey. Bul. 74, Bureau of Soils, U. S. Dept. Agr. (1910).

In addition to those here mentioned, a number of others are already isolated and will be reported upon in later publications from this laboratory. The methods by which the above compounds were obtained and the full description of their chemical properties are given in Bulletins 53 and 74 of this bureau. It is obvious that definite chemical information of this kind sheds much light upon the nature of soil organic matter and the processes going on in soils, but it is not the purpose of the present bulletin to discuss this question.

PURPOSE OF THE PRESENT INVESTIGATION.

While the work along the lines already laid down in the isolation and identification of definite soil constituents was progressing, it seemed desirable, if not imperative, that an idea be gained of the extent to which such constituents are distributed in the soils of the United States. Such information is necessary for a well-balanced opinion on the nature of soil organic matter in general, and for the recognition of the abnormal characteristics of organic matter in certain soils; that is, an ability to distinguish between what is normal and what is abnormal. A survey of a large number of soils from widely different regions was, therefore, made and a method devised for such examinations.

This method consisted in the coordination of the separate methods used in isolating these compounds, so as to form a more or less rigid scheme of procedure for treating a relatively small amount of soil. The investigation methods naturally involved the use of very large amounts of soil, sometimes many hundred pounds, amounts too large for a practical handling of so extensive a survey as was contemplated in this work. A further motive in formulating a rigid scheme of examination applied to a reasonably small sample of soil was to encourage such work among other investigators and students of the soil with a view to extending this work over a larger area with a greatly increased force, to the end that a most comprehensive survey of the occurrence of such constituents in soil may be had and that exact

Outline for the isolation of organic soil constituents.

- 1. Soil extracted with 2 per cent NaOH.
  - 2. (*a*) Acidify three-fourths of extract with HNO_3.
    - 3. Precipitate.
      - 4. Soluble in alcohol.
        - Soluble in water.
        - 5. Insoluble in water.
          - 6. Soluble in petroleum ether.
            - 7. Insoluble in cold alcohol. Saponify.
              - 9. Petroleum ether solution contains .... Hentriacontane. Agrosterol. Phytosterol.
              - 10. Residue contains .... α-Mono-hydroxystearic acid. Lignoceric acid.
            - 8. Soluble in cold alcohol. Precipitate with Pb acetate.
              - 11. Precipitate contains .... Paraffinic acid.
              - 12. Filtrate contains .... Liquid glycerides.
          - Insoluble in petroleum ether .... Resin acids. Resin esters.
      - Insoluble in alcohol.
    - 13. Acid filtrate. Extract with ether.
      - 14. Ether extract contains .... Dihydroxystearic acid.
      - 15. Neutralized aqueous solution.
        - 16. (*a*) Precipitated by Pb acetate.
          - Precipitate.
          - 17. Filtrate. Make alkaline with ammonia.
            - 18. Precipitate decompose with H_2S; add alcohol.
              - 19. Precipitate contains .... Pentosans.
              - 20. Filtrate contains .... Pentose sugars.
            - Filtrate.
        - 21. (*b*) Precipitated by $AgNO_3$.
          - 22. Precipitate contains .... Picoline carboxylic acid.
          - 23. Filtrate. Make alkaline with ammonia.
            - 24. Precipitate contains .... Cytosine.
            - Filtrate.
              - 28. Precipitate. Treat with HNO_3.
                - 29. Precipitate contains .... Xanthine.
                - 30. Filtrate contains .... Hypoxanthine.
              - Filtrate.
        - 25. (*c*) Precipitated in alkaline solution with Fehling's solution and dextrose.
          - 26. Precipitate.
            - 27. Filtrate. Add ammoniacal silver nitrate.
            - Precipitate.
          - Filtrate.
  - 31. (*b*) Acidify one-fourth of extract with H_2SO_4.
    - 32. Precipitate.
    - 33. Acid filtrate. Precipitate with phosphotungstic acid.
      - Filtrate.
      - 34. Precipitate. Decompose with Ba $(OH)_2$, etc.
        - 35. 37. Filtrate. Add solid Ba $(OH)_2$.
          - Filtrate.
          - 36. Precipitate contains .... Arginine.
        - Precipitate contains .... Histidine.

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knowledge of the nature of organic matter in soils might be increased and stimulated. Ten pounds of soil were decided upon as a sample of reasonable size and the accompanying scheme was devised.

SCHEME FOR THE ISOLATION AND IDENTIFICATION OF ORGANIC SOIL CONSTITUENTS.

In carrying out the work according to the accompanying scheme it will be necessary to take into consideration many minor operations or details which it is either impossible or impracticable to include in an outline like the above. The methods which have been selected for the isolation and identification of the compounds here considered had been devised for their isolation from plants and flesh, etc., where the interfering factors are fewer. When these methods of isolation are applied to soils, where both the mineral and organic matter are very complex, the interfering compounds are numerous and occur in such quantities at times that the compounds sought are often completely hidden. On further purification, which is sometimes almost impossible, the amounts of pure compounds obtained are extremely minute. Bearing these facts in mind, it at once becomes apparent that a thorough knowledge of the chemistry, both of the compounds to be isolated and their allied compounds, is necessary before such an investigation can be successfully undertaken. The investigator should be not only familiar with biochemical and organic methods of research, but he must also consult the original papers dealing with the isolation and properties of the various compounds sought. In this connection Bulletins 53 and 74 should be consulted, but for the guidance of the investigator the separate steps in the scheme of isolation will be briefly discussed here. The numbers refer to the separate operations shown diagramatically in the chart.

(1) Ten pounds of soil sifted through a 2-millimeter sieve to remove stones, roots, etc., are treated in a stoneware jar with 15 liters of a 2 per cent solution of pure sodium hydroxide for 48 hours with frequent stirring. The supernatant alkaline extract is siphoned off and (2) about three-fourths of the extract is acidulated with nitric acid. Nitric acid is used in this portion because of the subsequent use of lead and silver salts as precipitants. Acetic acid may be used, but some difficulty is experienced in the precipitation with silver salts since silver acetate is rather insoluble. Care must be taken that the nitric acid used be free from lower oxides of nitrogen and that only a slight excess of the acid is added, since a large excess would interfere in one or two operations to be explained later. The "humus" precipitate should be well flocculated and should settle out, leaving a clear solution. (3) The precipitate is collected on filter paper and washed until free from acid, and while still moist it is extracted with boiling alcohol until a color is no longer imparted to alcohol. (4) The alcohol solution is evaporated, keeping the total volume about the same by the addition of water to replace the evaporating alcohol. The portion of the humus insoluble in alcohol is discarded in the present scheme. The solution after the alcohol has been evaporated is filtered and the filtrate is discarded in this investigation. The residue insoluble in water (5) is dried, powdered, and extracted in a suitable manner with petroleum ether until all soluble matter has been removed; the

residue insoluble in petroleum ether contains the resinous compounds, resin acids, and resin esters described in Bulletin 74. (**6**) After evaporation of the petroleum ether the residue is dissolved in hot alcohol and the alcohol solution is allowed to cool; the portion insoluble in cold alcohol (**7**) is saponified with alcoholic potassium hydroxide, evaporated to dryness, and extracted with petroleum ether. (**9**) The petroleum ether solution is filtered and evaporated; the residue will contain hentriacontane,[1] agrosterol,[2] and phytosterol,[3] if present, which may be separated and recognized according to the methods described in Bulletins 53 and 74.

(**10**) The portion of (7) insoluble in petroleum ether is taken up in water, acidified to liberate the fatty acids and shaken out with ether. The ether extract will contain α-monohydroxystearic[4] and lignoceric[4] acids if they are present. They may be distinguished from each other by the methods described in Bulletin 74.

(8) The cold alcoholic filtrate from (6) is precipitated with alcoholic lead acetate and filtered and the precipitate (**11**), after being freed from lead salts by washing with alcohol, is suspended in alcohol and decomposed with hydrogen sulphide. The alcohol solution after being freed from hydrogen sulphide by boiling contains paraffinic[5] acid which may be further purified and identified by its melting point and analysis.

(**12**) The filtrate from (8) is freed from lead salts by treatment with hydrogen sulphide and on evaporation liquid glycerides, if present, will be obtained.

(**13**) The filtrate from (2) is shaken out with ether and (**14**) the ether solution evaporated off in a beaker over about 75 c. c. of water. The water solution may contain besides dihydroxystearic acid, nitric acid and other compounds soluble in ether. The nitric acid is eliminated by repeated washing of the ether solution with water and subsequent evaporation of the ether, as before, over water. The water is boiled and concentrated to a small volume and filtered while hot through a wet filter paper. This separates the dihydroxystearic acid, which is soluble in hot water, from any oily and resinous compounds insoluble in water. On cooling and standing for several hours the dihydroxystearic acid[6] separates out in starlike clusters of leaflets which can be identified by the microscope and by their melting point.

(**15**) The solution which has been extracted with ether is neutralized with sodium hydroxide and filtered. The precipitate is saved for treatment under (25). The neutral filtrate is divided into three aliquot portions. One of them (**16**) is precipitated with lead acetate, which removes much coloring matter and the precipitate discarded. To the filtrate (**17**) ammonia is added until a slight alkaline reaction is obtained and the solution filtered. (**18**) The filtrate is decomposed with hydrogen sulphide, filtered, and boiled down to small volume, and after cooling is added to three volumes of alcohol. After standing over night the gelatinous precipitate (**19**) is filtered off, washed with alcohol, and then tested for pentosans as follows: Test No. 1. A portion of the precipitate is boiled with a little concentrated hydrochloric acid and a few crystals of orcin. If pentosans[7] are present a green color is produced. It is a good plan to add a drop of dilute ferric chloride, since it assists in the quicker development of the color. After a strong color has developed amyl alcohol is added, which absorbs the color formed by the reaction of the orcin and the pentosans. Test No. 2. Some of the precipitate is boiled for a minute with concentrated hydrochloric acid and then a solution of phloroglucin in hydrochloric acid is added. If a red color is formed, changing to a violet red, which rapidly darkens due to a fine black precipitate, the presence of pentosans is indicated.

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[1] Paraffin Hydrocarbons in Soils, Oswald Schreiner and Edmund C. Shorey. Jour. Am. Chem. Soc., **33**, 81 (1911).

[2] The Presence of a Cholesterol Substance in Soils; Agrosterol, Oswald Schreiner and Edmund C. Shorey. Jour. Am. Chem. Soc., **31**, 116 (1909).

[3] Cholesterol Bodies in Soils; Phytosterol, Oswald Schreiner and Edmund C. Shorey. Jour. Biol. Chem., **9**, 9 (1911).

[4] Some Acid Constituents of Soil Humus, Oswald Schreiner and Edmund C. Shorey. Jour. Am. Chem. Soc., **32**, 1674, 1677 (1910).

[5] Bul. Soc. Chem., **23**, 111 (1875).

[6] The Isolation of Dihydroxystearic Acid from Soils, Oswald Schreiner and Edmund C. Shorey. Jour. Am. Chem. Soc., **30**, 1599 (1908).

[7] Chemical Nature of Soil Organic Matter, Oswald Schreiner and Edmund C. Shorey. Bul. 74, Bureau of Soils, U. S. Dept. Agr., 1910. Pentosans in Soils, Edmund C. Shorey and Elbert C. Lathrop. Jour. Am. Chem. Soc., **32**, 1680 (1910).

(20) The alcoholic filtrate is evaporated and taken up in a small volume of water, hydrochloric acid is added, and tests Nos. 1 and 2 are made as above; the green and red colors produced show the presence of pentose sugars.

(21) A second portion of the neutral filtrate (15) is precipitated with silver nitrate and filtered. The precipitate **(22)** formed removes most of the color from the solution. It is washed well to remove soluble silver salts, suspended in water, and decomposed with hydrogen sulphide. The solution is boiled to remove the excess of hydrogen sulphide, filtered, and concentrated. If the filtrate is very dark, reprecipitation with silver nitrate will be necessary. On evaporation to small volume and subsequent standing picoline carboxylic acid,[1] if present, will crystallize out and can be definitely identified by the method described in Bulletin 53.

(23) To the filtrate from (21) dilute ammonium hydroxide is added until precipitation ceases, care being taken not to add an excess. The solution is filtered and the precipitate **(24)**, after washing carefully with water, is suspended in water and decomposed with hydrogen sulphide, the solution is boiled to remove the excess of hydrogen sulphide, filtered, concentrated, and allowed to stand. Cytosine,[2] if present, will separate out on cooling in shining leaves or scales. It may be purified by reprecipitation as above and identified by its crystalline form, by the various characteristic crystalline salts which it forms, and by analysis.

(25) The neutralization precipitate from (15) is added to the third portion of the neutralized filtrate and the whole made strongly alkaline with sodium hydroxide and filtered. To the boiling filtrate Fehling's solution is added, together with a little dextrose. The solution after the precipitation is complete is filtered while hot and the filtrate is discarded in this scheme. The precipitate **(26)** is washed until neutral and after being suspended in water is decomposed in boiling solution with hydrogen sulphide; boiled to remove excess of hydrogen sulphide, and filtered. If the filtrate is not clear, the following steps must be taken: The solution is evaporated to dryness on the water bath, alcohol is added, and the solution again evaporated. The residue is taken up in hot water and filtered while still hot. This treatment generally precipitates the suspended copper sulphide. If it does not, a very little dilute nitric acid may be added. The filtrate **(27)** is allowed to cool, is made ammoniacal, and ammoniacal silver nitrate added until precipitation ceases. If xanthine and hypoxanthine are present, they will be precipitated as xanthine silver and hypoxanthine silver. The silver salts are collected on a filter and washed with water. The filter and precipitate **(28)** are placed in an evaporating dish and boiled for a minute with nitric acid, specific gravity 1.1, filtered hot, washed with the same strength of acid, and allowed to cool. The hypoxanthine silver nitrate formed crystallizes out after several hours and is filtered off. To the filtrate **(29)** ammonium hydroxide in excess is added and the precipitate formed is collected on a filter, washed well with water, and, after suspension in water, is decomposed with hydrogen sulphide, the excess of the latter removed by boiling, and the xanthine[3] identified by color reactions and characteristic salts, as described in Bulletin 74. The precipitate **(30)** is washed until the washings give a neutral reaction, suspended in water, and decomposed with hydrogen sulphide; excess of hydrogen sulphide is expelled, slight excess of ammonia added, the solution warmed to remove excess of the latter, and after filtering concentrated to small volume. The solution is then allowed to stand in a cool place when the hypoxanthine should crystallize out in small colorless needles. The identification of hypoxanthine[1] is described in Bulletin 74.

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[1] The Isolation of Picoline Carboxylic Acid from Soils and Its Relation to Soil Fertility, Oswald Schreiner and Edmund C. Shorey. Jour. Am. Chem. Soc., 30, 1295 (1908).

[2] Bul. 74, Bureau of Soils, U. S. Dept. Agr., 1910. Pyrimidine Derivatives and Purine Bases in Soils, Oswald Schreiner and Edmund C. Shorey. Jour. Biol. Chem. (8), 388 (1910).

[3] Pyrimidine Derivatives and Purine Bases in Soils, Oswald Schreiner and Edmund C. Shorey. Jour. Biol. Chem., 8, 391 (1910).

The second portion (**31**), consisting of one-fourth of the alkaline extract of the soil, is made rather strongly acid with sulphuric acid. The "humus" precipitate (**32**) so obtained after thorough washing may be added to the "humus" precipitate obtained from (2) and the filtrate may be shaken out with ether as described in (13) and the ether extract added to (14). After such treatment the filtrate (**33**) is precipitated with a solution of phosphotungstic acid and allowed to stand for several days.

The precipitate (**34**) thus formed is collected on a filter. The filtrate in this investigation was always discarded. The precipitate after being washed thoroughly with 5 per cent sulphuric acid is decomposed with an excess of barium hydroxide solution and filtered. The filtrate is acidulated with dilute nitric acid and silver nitrate added until a drop of the solution added to a saturated solution of barium hydroxide causes a yellow color to persist. Barium hydroxide is then added to slight alkalinity and the solution filtered. To the filtrate (**35**) solid barium hydroxide is added to strong alkaline reaction and the precipitate thus formed is collected on a filter paper. If no precipitate is formed, a little more silver nitrate should be added. The precipitate (**36**) is washed with barium hydroxide solution and suspended in water, acidulated with sulphuric acid, and decomposed with hydrogen sulphide. The solution is boiled to expel the excess of hydrogen sulphide and filtered from the silver sulphide formed. The filtrate is made slightly alkaline with barium hydroxide and the excess of barium hydroxide removed by means of carbon dioxide. The solution is boiled until all the carbon dioxide is removed, filtered, and evaporated to a small volume. Nitric acid is added and the solution allowed to stand. Arginine,[2] if present, will crystallize out as arginine nitrate in characteristic form. It may be identified as described in Bulletin 74.

The precipitate (**37**) is washed well with barium hydroxide, suspended in water, acidulated with sulphuric acid, and decomposed with hydrogen sulphide. The excess of hydrogen sulphide is expelled by boiling, the solution is filtered, and made alkaline with barium hydroxide; the excess of the latter is precipitated with carbon dioxide and when precipitation is complete the carbon dioxide expelled, and the solution filtered and evaporated to rather small volume. Ammoniacal silver nitrate is added to the filtrate until precipitation is complete, carefully avoiding an excess. The precipitate is collected on a filter, washed well with water, and decomposed with dilute hydrochloric acid. After filtering off the silver chloride the solution is evaporated to small volume and allowed to stand, when, if present, histidine[1] will crystallize out in the very characteristic form of histidine dichloride. For further identification see Bulletin 74.

Owing to the smallness of the soil sample the amount of "humus precipitate" obtained in some soils is so small that no adequate examination is possible by this part of the scheme. Another difficulty encountered is that the acid filtrate must be divided into several portions. This still further decreases the amount of material for any one separation. This often leads to a failure to find a substance even if it exists in the soil. The amount of a substance obtained may be so small that extreme purification is out of the question, and therefore in such cases, where distinct crystalline form or characteristic tests are not available, the identification becomes uncertain, as neither melting point nor analysis can be made.

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[1] Pyrimidine Derivatives and Purine Bases in Soils, Oswald Schreiner and Edmund C. Shorey. Jour. Biol. Chem., 8, 392 (1910).

[2] The Presence of Arginine and Histidine in Soils, Oswald Schreiner and Edmund C. Shorey. Jour. Biol. Chem., 8, 381, 383 (1910).

Failure to find any one of the compounds can not, therefore, be construed to mean that it is entirely absent. With some of the constituents, like dihydroxystearic acid or histidine, the result is much more definite. With dihydroxystearic acid the procedure is direct and the entire acid filtrate is used in its extraction with ether, involving no chance of loss through chemical treatment. Where dihydroxystearic acid is not found, therefore, when the extract from a 10-pound sample is used, it must mean that the compound is absent or, if present, in traces only. Experience has shown, therefore, that for the best results in searching for a number of compounds not less than 100 pounds of soil should be used, unless the soil be very high in organic matter, or a specific test applied, or the experimenter exceptionally experienced in the isolation.

As has already been mentioned, the scheme is an adaptation and coordination of the methods of isolating from different soils the single compounds dealt with here. In the application of the scheme to different soils it has been found that for each soil some few details in one part or other of the scheme must be more or less modified. This modification is made necessary by the fact that the organic material in different soils is really different, so that the particular compound occurs under different associations, which often render a change in the method of isolation, identification, or purification absolutely necessary. The experience and judgment of the investigator must in a large measure determine what modification to make. When a substance is isolated and is not identical with the soil constituent already found at this place in the scheme, it may nevertheless belong to the same class of compounds as the constituent already identified. In this manner new compounds may be discovered in the course of such an investigation. Some of the filtrates and precipitates in the scheme are discarded because as yet they have yielded no definite compounds. However, these portions may be examined for such compounds as the investigator sees fit. They will no doubt also contain, because the question of solubility is only a relative one, some or all of the compounds discussed above.

The portions designated for the final isolation and identification of the various compounds will almost always be contaminated with coloring matter, resinous material, and other bodies precipitated by the same metallic salts or soluble in the same solvents, and such foreign material must, of course, be eliminated by careful reprecipitation or by repeated solution. The metallic salts selected for this investigation as precipitants do not in most cases completely precipitate the compounds with which they combine, but have been chosen because they seemed to be the most adequate for the purpose. The compounds which they precipitate are often precipitated by other metallic salts used in the scheme; for instance, cytosine, while isolated here by the use of ammoniacal silver nitrate, is also precipitated to some extent by ammonical lead acetate. Care must be taken not to confuse crystals of calcium sulphate with the crystals of the hydrochlorides or other salts of the purine bases, for it has been our experience that calcium sulphate often appears in the final solution to be used for the identification of these compounds. Since phosphotungstic acid is a general precipitant for organic bases, some of which may also be precipitated by the other metallic salts used in the isolation of the hexone bases, care must be exercised that salts of other organic bases are not confused with the crystalline salts of arginine and histidine.

RESULTS OF THE INVESTIGATION.

The results obtained on a number of samples from different localities by the application of the scheme outlined for the separation and identification of definite organic constituents in these soils are briefly given in the following paragraphs, the soils being grouped according to the States in which the samples were collected:

Maine.—Two samples of Orono clay from the vicinity of Oldtown, Me. In both samples were found pentosans, xanthine, and histidine, and in one of the samples there was found, in addition, hypoxanthine.

Massachusetts.—Five samples from various parts of Boston Common, Boston, Mass. All of the samples showed the presence of pentosans, cytosine, hypoxanthine, and dihydroxystearic acid.

New York.—A sample of Volusia silt loam from Ontario County, N. Y., showed the presence in the surface soil of pentosans, cytosine, and histidine, and in the sample of subsoil there was found in addition to these compounds hypoxanthine and dihydroxystearic acid.

A sample of Clyde loam from Orleans County, N. Y., contained in the surface soil pentosans, pentose sugars, histidine, cytosine, hypoxanthine, and dihydroxystearic acid. The sample of subsoil showed in addition to the above compounds the presence of xanthine.

The examination of a sample of muck soil from Orleans County, N. Y., showed in upper as well as lower layers of the muck the presence of pentosans, pentose sugars, and histidine.

Pennsylvania.—A sample of Chester silt loam from the vicinity of Philadelphia, Pa., showed the presence of agroceric acid, pentosans, cytosine, arginine, xanthine, hypoxanthine, and dihydroxystearic acid.

A sample of Frankstown stony loam from Bedford County, Pa., contained in the surface soil pentosans, pentose sugars, histidine, and dihydroxystearic acid. The sample of subsoil, however, did not show the presence of the dihydroxystearic acid, but did show the presence of the other compounds found in the surface soil.

Maryland.—In a sample of Elkton silt loam from the vicinity of Easton, Md., was found paraffinic acid,[1] α-monohydroxystearic acid,[1] pentosans, xanthine, and dihydroxystearic acid.

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[1] Bul. 74, Bureau of Soils, U. S. Dept. Agr., 1910.

West Virginia.—Samples of both surface and subsoil of Dekalb silt loam from the vicinity of Ravenwood, W. Va., showed the presence of pentosans, pentose sugars, and histidine.

Kentucky.—A sample of Clarksville silt loam from the vicinity of Broadhead, Ky., on examination showed the presence of pentosans, pentose sugars, histidine, and dihydroxystearic acid in the surface soil. In the subsoil the same compounds were found with the exception of dihydroxystearic acid.

North Carolina.—Investigation conducted on a peat soil from the region bordering Lake Mattamuskeet, N. C., revealed the presence of hentriacontane, lignoceric acid, phytosterol, several resin acids, and resin esters reported in Bulletin 74.

Mississippi.—Investigation of a sample of Houston clay from Noxubee County, Miss., showed it to contain histidine and arginine, as reported in Bulletin 74.

Texas.—A sample of Amarillo silt loam from Sherman County, Tex., showed the presence of pentosans, pentose sugars, and histidine in both surface and subsoil.

North Dakota.—A sample of Marshall clay from Fargo, N. Dak., under investigation showed the presence of agrosterol, agroceric acid, xanthine, and hypoxanthine, as recorded in Bulletins 53 and 74.

In discussing the foregoing results it is not intended to dwell upon the origin of these substances in the soils examined. Suffice it, therefore, merely to recall here the facts more fully discussed in Bulletins 53 and 74, that soil organic matter consists of the débris of all plant and animal residues and that the individual constituents of soil organic matter comprise (1) the accumulated substances not readily subject to change, existing in the same forms as in the original plant or animal material, and (2) all the decomposition products which would arise through any agency whatsoever from proteins, fats, lecithins, carbohydrates, alkaloids, terpenes, and all other constituents of previous vegetation or animal occupation.

The above list comprises 26 samples from 11 States. From 23 of these soils pentosans were isolated; the other 3 had not been subjected to this examination. Two of these soils, however, indicate large amounts of pentosans to be present by the furfurol test, these two soils being the Marshall clay and the North Carolina peaty soil, as already reported.[1] It would appear, therefore, that pentosans are general constituents of soil organic matter.

Pentose sugars were found in 11 of the soils. The test for pentose sugars was not made on the earlier samples examined, but it was found in all samples on which a test was made. It would appear, therefore, that pentose sugars or pentose-containing complexes—not pentosans—the chemistry of which will be reported on in later publications, are common constituents of soils.

Histidine is a primary decomposition product of protein material. Its occurrence in 17 soils of the 24 examined for it indicates that this hexone base is a fairly common constituent of soils and shows, therefore, that the protein cleavage in the soil is similar to that brought about by chemical or other action in laboratory investigations. That histidine persists in the soil during and after protein decomposition may be due to the fact that it is more resistant to change than other compounds of similar nature and origin.

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[1] Pentosans in Soils, Edmund C. Shorey and Elbert C. Lathrop. Jour. Am. Chem. Soc., **32**, 1680 (1910).

Arginine is a common cleavage product of protein, but has so far been found in only two soils and is probably not a soil constituent which persists for any length of time.

Cytosine is derived from the breaking down of nucleo-proteids and nucleic acids existing in all plant cells. The fact that it was found in 10 soils out of 23 examined indicates that it also may be a rather common soil constituent and shows conclusively that the change of such constant complex plant constituents as nucleic acid progresses in the same manner as it does outside of the soil through chemical agencies better understood.

Xanthine and hypoxanthine exist in plants as such and like cytosine also result from the cleavage of nucleic acids and nucleo-proteids. They further result from other purine bases, guanine and adenine. It is, therefore, not possible to state at this time the direct source of the xanthine compounds in the soils. It is probable that their occurrence will be traced to all these sources. Out of the 24 soils examined for these purine compounds, 5 contained xanthine and 9 contained hypoxanthine, thus showing that these purine bases will probably be frequently encountered in soil work of this character. Some of the soils contained xanthine only and some hypoxanthine only. Four of the soils contained both. Hypoxanthine is more resistant to bacterial action and this may explain its more frequent occurrence in the soils examined.

Although agroceric acid, lignoceric acid, paraffinic acid, α-monohydroxystearic acid, as well as agrosterol, phytosterol, and hentriacontane are reported as having been found only once or twice in the above list of soils, no general statement covering their frequency or lack of frequency of occurrence is warranted. This, as already pointed out, is really due to the fact that the amount of material obtained in the course of the scheme here employed was too small for a satisfactory examination for these constituents. In fact, the use of other methods of procedure, such as the direct extraction of soils with alcohol, indicates that some of the constituents are probably much more common than the above results appear to show.

Dihydroxystearic acid will be discussed more fully in regard to its occurrence and other properties in the succeeding pages, but some remarks on its occurrence in the above soils should here be made, especially in connection with its association with other constituents. Eleven out of the 26 soil samples contained dihydroxystearic acid; 5 of these, however, were from a single locality. An interesting fact which may prove to be important is that in only one case was dihydroxystearic acid found without either hypoxanthine or xanthine or both being found at the same time. In seven of the samples it was accompanied by hypoxanthine only, in two samples by hypoxanthine and xanthine and in one sample by xanthine only.

DIHYDROXYSTEARIC ACID AS A SOIL CONSTITUENT.

Dihydroxystearic acid was found so often in the examinations of infertile soils brought to the attention of this laboratory as to lead to the conclusion that it is one of the principal factors of infertility in such soils or at least a contributory factor that is easily determined and thus becomes an indicator of the poor soil conditions which lead to infertility. More accurate knowledge concerning the other organic soil constituents associated with and accumulating under the same conditions as dihydroxystearic acid will doubtless be necessary before this question can be definitely answered, but attention should here be called to the fact that even the mere knowledge that this one constituent is present leads to a recognition of the fact that poor soil conditions exist, and this leads to the practical remedial measures to be employed. In other words, the practical treatment that will rid the soil of dihydroxystearic acid also corrects most of the other detrimental influences of poor soil conditions. The determination of the presence of this compound becomes, therefore, a very valuable and ready means of diagnosing such soil conditions on a small laboratory sample. A large number of samples were examined for this constituent and the results reported in this bulletin. Its frequent occurrence in particularly poor soils makes it desirable to present first the results of special studies on the properties of dihydroxystearic acid, its method of isolation, effects on plant growth, and the means which under field conditions will tend to eliminate it from the soil or minimize its injurious action.

PREPARATION AND PURIFICATION.

Dihydroxystearic acid can be prepared from the soils which contain it or in the chemical laboratory from oleic acid.

From soils.—The soil is treated with 2 per cent sodium hydroxide solution, stirred frequently and finally allowed to settle. The supernatant alkaline extract is made acid with a slight excess of sulphuric or other acid and filtered from the precipitate formed; the acid filtrate is shaken out with ether and the ether allowed to evaporate on the surface of a small quantity of water in a warm place. The ether on evaporating leaves on the surface of the water some colored resinous and oily material, together with nodules or concretions of a crystalline compound which is dihydroxystearic acid. On heating the water to boiling, the crystals go into solution while the resinous matter remains undissolved. On pouring the solution into a filter, previously wet with hot water, the resinous and oily material remains in the filter. On cooling the solution so obtained, crystals separate, generally in the form of small plates or leaflets arranged in small radiating clusters. These, if still colored, can be purified by solution in hot water and filtering as before.

From oleic acid.—Its preparation from oleic acid is by the method described by Saytzeff.[1] Oleic acid is treated for a few minutes at a low temperature with nitrous oxide generated in any convenient way, say by the action of nitric acid on arsenic trioxide, in order to change the oleic acid to the isomeric elaidic acid. This is washed with water and recrystallized from ether or alcohol. It should melt at 45–47° C. The elaidic acid is dissolved in a dilute solution of potassium hydroxide and treated with potassium permanganate. The elaidic acid is oxidized, dihydroxystearic acid being one of the products. The yield obtained in this way is low, the dihydroxystearic acid formed being easily oxidized further to other compounds. The alkaline solution of the oxidation products is decanted from the manganese oxides formed and is acidified with sulphuric acid. The precipitate formed is collected on a filter and the filtrate is shaken out with ether, the dihydroxystearic acid being somewhat soluble in cold water. The ether solution is evaporated over a small volume of water and the residue is added to the acid precipitate, collected as above described. As dihydroxystearic acid is much more soluble in hot water than in cold, it is purified by this means. The precipitate is dissolved in boiling water and filtered immediately. On cooling, the dihydroxystearic acid crystallizes out and by a repetition of this process the acid can be obtained in a pure condition.

PROPERTIES.

Dihydroxystearic acid is a white crystalline compound, slightly soluble in cold water, more readily soluble in hot and easily soluble in alcohol and ether. It crystallizes out of water in radiating clusters or concretions and when pure, in small plates or leaflets,[2] which are generally arranged in starlike groups. Its melting point is 98° to 99° C. The calcium and barium salts are easily formed by the decomposition of the corresponding carbonates. They are little soluble in cold water, but quite readily soluble in hot water. The barium salt crystallizes in somewhat characteristic circular concretions of radiating structure. The silver salt is amorphous and is quite insoluble in water.

In the preparation of dihydroxystearic acid from elaidic acid attention was called to the fact that the yield of dihydroxystearic acid was low. This is due to the further oxidation of the dihydroxystearic acid first formed. Edmed [1] in studying the oxidation products of oleic and elaidic acids with alkaline permanganate, obtained from the latter dihydroxystearic acid, 33 per cent; pelargonic acid, 13 to 14 per cent; azelaic acid, 26 per cent; and oxalic acid, 15 to 20 per cent. By further subjecting the dihydroxystearic acid obtained to the action of alkaline permanganate the dihydroxystearic acid was completely decomposed, pelargonic, azelaic and oxalic acids being the sole products of the oxidation. It would seem then that dihydroxystearic acid is an easily oxidizable compound and that any treatment that would tend to increase the oxidative power of a soil thus contaminated would likewise cause a diminution or entire disappearance of this compound.

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[1] Jour. prakt. Chem., **33**, 300 (1886); **50**, 76 (1894).

[2] Bul. 53, page 32, Plate II, Bureau of Soils, U. S. Dept. Agr., 1909.

POSSIBLE ORIGIN OF DIHYDROXYSTEARIC ACID.

Incidental to the present investigation it was hoped that the examination of a considerable number of soils would throw further light upon the origin of this compound. For this reason as much information concerning the crop history and native vegetation as could be acquired from the respective owners of the soils examined was obtained, but the information thus gained is so meager and imperfect that we hesitate at this time to draw any definite conclusions. It seems, however, that the dihydroxystearic acid is not connected with any specific crop and what information there is points to its association with soil fungi, as already pointed out. Some of the soils in which it has been found are well suited to the growth of fungi and the rootlets of plants growing in these soils were often infested with mold. A quantity of oak roots with mold attached taken from the Takoma soil in which the compound had been found was washed free from adhering soil and this root and mold examined for dihydroxystearic acid with the result that the presence of this compound was shown. Oak roots from another soil and locality not infested with mold did not give the substance.[2] This, together with the fact that some of the other soils, likewise, contained plants having mold-infested roots would seem to warrant the conclusion that such molds may produce this substance from the organic material which finds its way in one manner or another into the soil. The particular substances which furnish the basis for this formation of dihydroxystearic acid are not yet known and it is also possible that agents other than the fungi here suggested may produce it in the soil, such as its formation by oxidation of fats or similar decomposition of other organic compounds, such as protein material. The soil conditions under which it is found are generally poor drainage, poor aeration, too great compactness, deficiency in lime, lack of good oxidation, lack of good nitrification, and, as has already been mentioned, tendency for fungus development and exceptional poorness of crops. While, therefore, all these factors contribute toward the formation or accumulation of dihydroxystearic acid or exist because of its presence, no single one of these factors can, at the present time, be said to explain its origin wholly.

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[1] Jour. Chem. Soc., 73, 627 (1898).

[2] Bul. 53, Bureau of Soils, U. S. Dept. Agr., 1909.

EFFECT ON PLANT GROWTH.

Dihydroxystearic acid isolated from soils and also the compound prepared chemically has been fully tested in regard to its effects on plant development and growth. In this connection see Bulletins 53 and 70 for details, but the results may be briefly stated here.

When a soil containing dihydroxystearic acid was extracted in the manner described for its isolation and the ether solution evaporated and a solution of the residue in pure distilled water tested with plants, it was found to be very harmful and the harmful effect was proportional to the amount of the compound present.

The isolated and purified dihydroxystearic acid was likewise tested by dissolving it in pure distilled water and it was found to have decided deleterious action on the wheat seedlings used in the tests. The acid prepared in the laboratory behaved in the same manner.

Its effect in the presence of nutrient salts was also extensively studied in solutions containing calcium acid phosphate, sodium nitrate, and potassium sulphate, alone and in all combinations of two and three of these salts, a total of 66 cultures being used in a single test. The injurious effect of the dihydroxystearic acid was less where all three of the nutrient elements were present than where only one or two were present. The injurious effect was least in those cultures of three nutrient elements where the nitrate was high. This indicates that the action of the nitrates tends especially to overcome the harmful effect of the compound or else it enables the plant to resist or overcome its effect.

The effect of the compound on the wheat seedlings employed in these tests first shows itself in its action on the roots and later the tops also show its harmful influence. The roots become stunted in their growth and develop enlarged, much swollen ends, the extreme tips being much discolored, sometimes quite black, and often show the phenomenon of turning upward at the tip giving the roots a hooklike appearance. The tops show a diminished growth with thin stems, the leaves have a lighter green color than normal, and in later stages or in stronger solution become yellowish at the tips and show a generally weakened condition. The amount necessary to produce these effects is quite minute, 10 to 50 parts per million; with larger amounts death of the plant may result. These same effects are shown by plant roots and tops grown in soils containing dihydroxystearic acid and in water extracts prepared from such soils.

When nitrates are present in the fertilizer mixtures above mentioned, the characteristic effects on the roots and tops become less marked and may disappear altogether, the roots being long and white with good branching and the tops show a corresponding improvement in color and strength. This may be due to the fact that the normal oxidation of the roots is restored by the nitrates. Dihydroxystearic acid interferes greatly with the normal oxidation of roots and nitrates stimulate this oxidation. What becomes of the dihydroxystearic acid is not definitely known, but all indications point to the fact that it is ultimately oxidized to other compounds not harmful to plants.

EFFECT ON ABSORPTION OF PLANT NUTRIENTS.

Dihydroxystearic acid has another effect which should here be mentioned as having a considerable bearing on its effect on crops and on its occurrence even in such soils as contain much plant nutrient material in the most readily available form. This is its influence on the absorptive power of the roots of the plants growing in the soil, the soil solution or solutions of nutrient salts when dihydroxystearic acid is present in them. This influence has been thoroughly studied by analyzing the culture solutions mentioned in the previous section every three days during the growth of the seedlings.[1] When compared with the results obtained in similar cultures in which no dihydroxystearic acid was contained, it was found that the solutions with dihydroxystearic acid contained more nutrient materials, especially potassium and phosphate, than the solution in which the harmful compound was absent. In other words, the presence of the compound so affected the absorbing power of the roots that although nutrients were plentifully furnished, the plant made little use thereof, except of the nitrates. This is in harmony with the fact stated above that when nitrates were plentiful in the solutions, the best growth was obtained and the effect of the harmful compound was minimized or entirely overcome.

ELIMINATION AND PREVENTION.

Dihydroxystearic acid is readily changed by oxidation to other compounds. Its very existence in soils depends therefore upon poor oxidation or aeration in the soil, and this effect is remedied by such soil treatments as promote oxidation. When the soil samples containing the harmful compounds are kept in the laboratory or greenhouse under conditions of good aeration for a few weeks the compound disappears entirely and can be no longer detected by chemical means, the soil having also become greatly improved for plant growth through this treatment.

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[1] Some Effects of a Harmful Organic Soil Constituent, by Oswald Schreiner and J. J. Skinner, Bul. 70, Bureau of Soils, U. S. Dept. Agr. (1910).

Soils containing dihydroxystearic acid have usually shown a low oxidizing power, when treated with aloin according to the method described in Bulletin 73.[1] Liming, drainage, and the application of good organic manures are the most potent factors in promoting oxidation in soils. Liming has been found to be very beneficial, aiding the destruction of harmful bodies of this nature, both by combining with them and also by oxidizing them to other less harmful or even beneficial compounds. Drainage also aids materially in two ways: First, it allows a freer access of air with deeper penetration of healthy roots, which in time promotes oxidation and thus aids the destruction of unfavorable organic matter; second, it produces beneficial results by an actual removal of the harmful material in the drainage waters, as well as perhaps by eliminating the cause of its formation. The addition of good organic manures will also assist in the destruction of the compounds already in the soil. The introduction of easily decomposed organic matter causes greater bacterial activity and greater oxidation in the soil, so that through the inauguration of the processes of its own decomposition, it has the effect of causing the change in the organic matter in the soil to proceed in a different direction from that which formerly existed. The fertilizer salts are shown by experiments to act in a very similar manner, inducing or quickening changes which take place without them only very slowly or not at all.

EXAMINATION OF SOIL SAMPLES FROM WIDELY DISTRIBUTED POINTS FOR DIHYDROXYSTEARIC ACID.

The frequent occurrence of dihydroxystearic acid in the soil samples examined, together with the fact that it was found in soils unproductive in the field and the fact that the isolated compound had decidedly harmful action on growth and absorption of plant nutrients, made it seem highly desirable to test this relationship of soil infertility or low productivity with the presence of this compound, more fully. With this in view, samples were collected by field men of the Bureau. A good sample and a poorer sample of the same soil type, either in the same field or at least the same neighborhood, without necessarily any special problems being connected therewith, were requested, and in most cases this request could be complied with. In addition to those collected in this manner infertile soils

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[1] Studies in Soil Oxidation, by Oswald Schreiner, M. X. Sullivan, and F. R. Reid, Bureau of Soils, U. S. Dept. Agr. (1910).

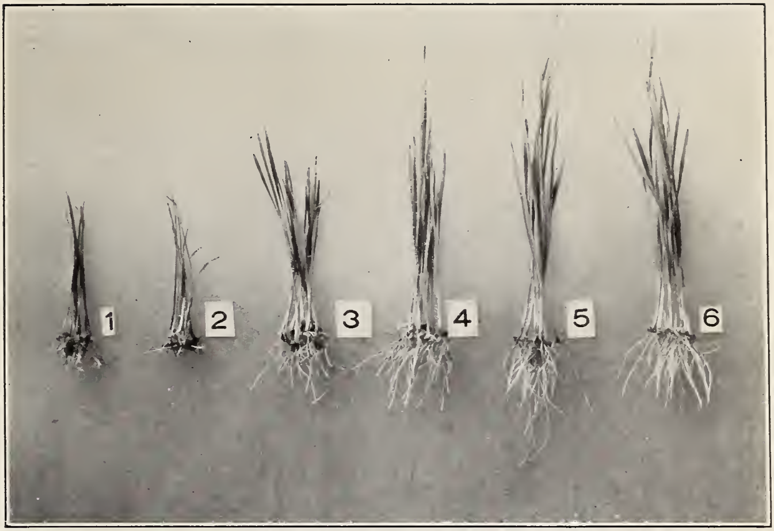

FIG. 1.—HARMFUL EFFECTS OF MATERIAL EXTRACTED BY ETHER IN THE COURSE OF ISOLATING ORGANIC SOIL CONSTITUENTS.

1 and 2, full strength; 3 and 4, half strength; 5 and 6, one-tenth strength.

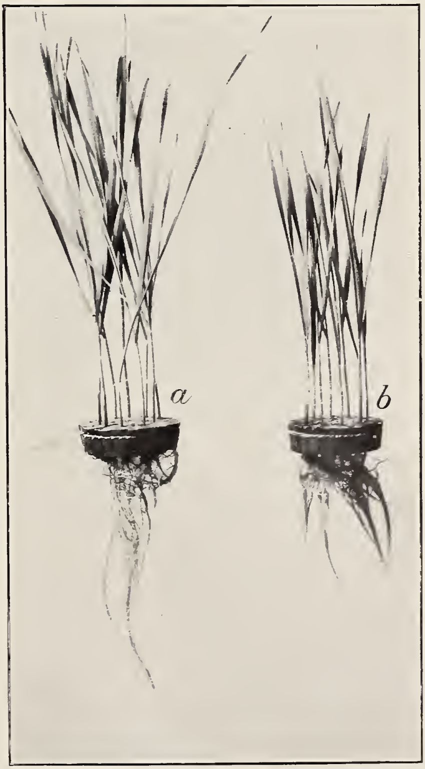

FIG. 2.—EFFECT OF DIHYDROXYSTEARIC ACID ON PLANTS IN A NUTRIENT SOLUTION.

a. Wheat seedlings in nutrient solution containing fertilizer salts.
b. The same as *a*, but with 50 parts per million dihydroxystearic acid added.

with a known record of infertility were examined. These samples of good and poor soils were examined for dihydroxystearic acid.

When an examination for dihydroxystearic acid alone in a soil is to be made, the method already described may be slightly changed to advantage. Five to ten pounds of soil is a sufficiently large sample. This is treated for 24 to 48 hours with 5 to 10 liters of 2 per cent sodium hydroxide solution. For the acidification of the alkaline extract sulphuric acid is used instead of nitric acid, because of the fact that sulphuric acid is not soluble in ether while nitric acid is more or less soluble. The ether extract is evaporated as described and the water solution of dihydroxystearic acid boiled and filtered through a wet filter paper to remove oily matter and resins. The extraction is again made with ether and the process of evaporation and filtration repeated. By this time from most soils the dihydroxystearic acid in solution is pure enough to be crystallized; in exceptional cases, however, the solution may require further purification. The purified water solution is evaporated to small volume, when, on standing, the dihydroxystearic acid will crystallize out, and may be identified in the usual manner.

The results of this examination for the occurrence of dihydroxystearic acid in good and poor soil samples from many parts of the United States, comprising soils of widely different texture, origin, cropping, climatic conditions, drainage conditions, etc., will now be given. For the sake of completeness the soils mentioned in a preceding section of this bulletin are also included in the following enumeration and discussion:

DESCRIPTION OF SOILS EXAMINED AND RESULTS OBTAINED.

Alabama.—No. 1. Norfolk loam, good, from Mobile County, Ala., a well-fertilized soil, gave no evidence of dihydroxystearic acid.

No. 2. Norfolk loam from the same vicinity, poorly managed and not fertilized, gave no indication of the presence of dihydroxystearic acid.

Nos. 3–4.—Louisa fine sandy loam from Roanoke, Ala.; samples from a good field and from a poor spot in the same field gave no indication of the presence of dihydroxystearic acid.

No. 5. Dekalb fine sandy loam, good soil, from Long Island, Ala., gave no dihydroxystearic acid.

No. 6. A sample from a poor field of the same soil type, devoid of humus and poorly cultivated, also gave no indication of dihydroxystearic acid.

No. 7. A sample of Orangeburg fine sandy loam from Tuscaloosa, Ala., from a good field gave no dihydroxystearic acid.

No. 8. A sample from a poorer field of the same type in this vicinity likewise showed no dihydroxystearic acid.

California.—Nos. 9–10. Madera sand from a good and from a poor field near Fresno, Cal., did not contain dihydroxystearic acid.

No. 11. Sample of soil from Stockton, Cal., did not show the presence of dihydroxystearic acid.

Connecticut.—Nos. 12–14. Samples of soil and subsoil from near Redding, Conn., were examined for Dr. Benj. C. Gruenberg, of the New York Botanical Gardens, who found that the soil was well adapted to the growth of *Corallorhiza.* This soil is not under cultivation, but supports a growth of timber and natural vegetation. The soil is high in organic matter, but by no means peaty. This soil was found to contain large amounts of dihydroxystearic acid. Dr. Gruenberg describes the sample of soil collected by him in the spring of 1909 as follows: "The soil occurs in pockets among rocks, the latter micaceous; there is considerable limestone in the vicinity. The trees are mostly oak and chestnut; a few maples, basswood, and pine. The ground is nearly bare of vegetation, even in the summer; small tufts of grass, some wintergreen, and several specimens of *Goodyera* (*Peranium*) were all I could find. The ground is covered with the leaves of last autumn."

In the winter of 1911 a larger sample of soil and of subsoil was collected by Mr. Frank Bennett of this bureau from the same locality as that collected by Dr. Gruenberg. This larger sample of soil was not collected from specific pockets, which was impossible on account of a blanket of snow. Both the surface and subsoil showed the presence of dihydroxystearic acid in considerable amounts, although it was not as plentiful as in the earlier sample. Nevertheless the amount obtained was large, being at the rate of 400 pounds per acre in these later samples.

Georgia.—Nos. 15–16. Surface and subsoil samples of Clarksville gravelly loam from Walker County, Ga., did not show the presence of dihydroxystearic acid. The soil is at present in timber, neighboring land is of moderate productivity.

Nos. 17–18. From samples of Cecil clay, surface and subsoil, from Appling, Ga., no dihydroxystearic acid was isolated. This is reported as a good soil.

Nos. 19–20. From samples of Cecil clay, surface and subsoil from a field adjoining No. 17, no dihydroxystearic acid was obtained. The field from which these samples were taken is not as productive as the above and is poorly drained.

Nos. 21–22. Samples of surface and subsoil of Norfolk fine sand from a good field in Glynn County, Ga., gave no dihydroxystearic acid when examined.

Nos. 23–24. Samples from a poor field of this same soil in the same locality likewise gave no positive result when tested for dihydroxystearic acid.

Nos. 25–26. Norfolk fine sand, samples of surface and subsoil from a good field near Savannah, Ga., gave no indication of the presence of dihydroxystearic acid.

Nos. 27–28. Samples of soil and subsoil from a poor field of Norfolk fine sand, one which had not been fertilized for some years past, likewise showed no indication of the presence of dihydroxystearic acid.

Kentucky.—Nos. 29–30. In surface soil and subsoil samples of Clarksville silt loam from Broadhead, Ky., dihydroxystearic acid was found in the surface soil, but not in the subsoil. This soil is not considered productive unless fertilizers are used.

Maine.—No. 31. Orono clay, near Oldtown, Me. An average productive soil, has received some manure, drainage could be improved. No dihydroxystearic acid found in this soil.

No. 32. Sample from poor spot in field from which sample No. 31 was taken. In this sample, likewise, dihydroxystearic acid was not found.

Maryland.—No. 33. A sample of surface soil of Elkton silt loam from near Easton, Md., was found to contain dihydroxystearic acid. The field from which this soil was taken is very unproductive. This and the following sample of Sassafras silt loam were sent in by Mr. H. H. Bennett of this bureau for special examination and are good examples of different fertility being shown by two soils that have been derived from the same material (Coastal Plain deposits) and which are similar in texture. They are different in that one is considered poor while the other is a very desirable type. "Both the Elkton silt loam and the Sassafras silt loam were taken from the same field and the accompanying pictures show the relative growth of corn on each of those soil types. Although the corn over the entire field received about the same treatment, that on the Elkton silt loam is very poor while that on the Sassafras silt loam is good. Both soils have received applications of seven or eight loads of barnyard manure per acre, applied to the surface of the sod, and the sod and manure then plowed under.

"The sample of Elkton silt loam was taken to a depth of 9 inches; the color varies from light gray at the surface to nearly white in the lower portion. The soil tends to run together during rains and compacts and makes cultivation difficult with subsequent dry weather. Close observation reveals in certain strata of this sample a kind of sponginess caused by the presence of small globular cavities. The lower portion of the soil is more or less laminated; clods are formed, but are broken rather easily. Along the line of contact of the soil and small rootlets there is a reddish-brown tinge. The manure at a depth of 4 or 5 inches has not yet thoroughly rotted, some of it retaining almost the original structure and strength of fiber. The subsoil from 8 to 16 inches is a mottled (reddish yellow and greenish gray) silt loam, carrying considerable clay; from 18 to 36 inches a lighter colored silt loam; and below, a dark-gray, rather plastic, silty clay, carrying some coarse sand below 40 inches."

No. 34. In a sample of surface soil of Sassafras silt loam from near Easton, Md., no dihydroxystearic acid was found. This soil is in good condition and is considered a comparatively productive soil. "This soil was likewise taken to a depth of 9 inches from the same field as the above Elkton silt loam, about 200 yards from the place where the former soil was collected. It is quite uniform in color, texture and structure, being yellowish-brown, friable silt loam. The manure at a depth of from 4 to 5 inches is thoroughly decomposed, a condition which did not exist in the Elkton silt loam. The soil tends to run together and harden, though probably not so much as in case of the Elkton silt loam. The content of moisture appears about the same as in the Elkton silt loam. The subsoil from 9 to 28 inches is a uniform reddish-yellow, fairly friable silt loam. Below 28 inches it is compact and lighter, varying from a heavy silty loam to a silty loam."

No. 35. A sample of Takoma lawn soil from Takoma Park, Md., was found to contain dihydroxystearic acid. This was a very infertile soil and did not respond either to stable manure or fertilizers. For a further description of this soil see Bulletins 28 and 53.

No. 36. A sample of peat from Lanham, Md., was submitted by Dr. F. V. Coville of this department and was found to contain dihydroxystearic acid. The peat "consists of dark-brown turfs or mats, 2 to 4 inches thick, made up of partially decomposed leaves interlaced with fine roots. It is found in thickets of the American laurel (*Kalmia latifolia*) where the leaves of this shrub, usually mixed with those of various species of oak, have lodged year after year and the accumulated layers have been partly decayed." Fungus growth is evident in the peat. For a more detailed description of this peat and its properties the reader is referred to the bulletin on "Experiments in Blueberry Culture," by Dr. Coville.[1]

Massachusetts.—Nos. 37–41. These samples were examined in connection with the plans for the improvement of Boston Common. Three different surface soils and two subsoils from various parts of the Common were examined and all were found to contain dihydroxystearic acid in fair amount, both in surface soils and in the subsoils. These samples were sent in for special examination, as the Common was suffering from impoverished lawns and trees.

Mississippi.—No. 42. A sample of Orangeburg fine sandy loam from a good field near Waynesboro, Miss., did not show the presence of dihydroxystearic acid.

No. 43. A sample of Orangeburg fine sandy loam from a field less productive than the above did not show the presence of this compound.

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[1] Bul. 193, Bureau of Plant Industry, U. S. Dept. Agr., 1910.

Nos. 44–45. Samples of Orangeburg sandy loam from a good field as well as from a poorer field in Forrest County, Miss., did not show the presence of dihydroxystearic acid.

Nos. 46–47. Samples of Cahaba fine sandy loam from a good and from a poorer field from Lowndes, Miss., did not show any dihydroxystearic acid.

New Jersey.—Nos. 48–49. Samples of surface soil and of subsoil of Dutchess loam from the vicinity of Greenville, N. J., were found to contain no dihydroxystearic acid. This soil is in a good state of productivity and has good drainage.

New York.—Nos. 50–51. An examination of samples of surface and subsoil of Volusia silt loam from a very poor field in the vicinity of Naples, N. Y., did not reveal the presence of dihydroxystearic acid in the surface soil, but it was found in the subsoil. The sample was collected by M. E. Carr of this bureau and was taken from "three different points in a field that had been cleared and cultivated for many years, with the usual results for this type of soil, viz, almost abandoned farm land. This field was plowed four years ago and gave a poor crop of oats and a poor seeding, there being almost no clover. Fourteen acres gave a crop of 3 tons of very poor quality of hay, or less than one-fourth ton per acre. No manure or commercial fertilizer were used." For the special problems connected with soils of this series see Bulletin 60 of this bureau.[1]

Nos. 52–53. In samples of Clyde loam from Orleans County, N. Y., dihydroxystearic acid was found in both the surface and the subsoil. This field is poorly drained, but has supported a good sod of wild grasses.

Nos. 54–55. Samples of muck soil from Orleans County, N. Y., both from the upper and lower strata, contained no dihydroxystearic acid. This soil has never been plowed, but supports a good growth of wild grasses. It is flooded in the spring, but is dry in the fall.

Nos. 56–57. Samples of Dunkirk clay, both soil and subsoil, from Monroe County, N. Y., were found to contain dihydroxystearic acid. This is a soil of fair productivity, with a very compact, tenacious, chocolate-colored clay subsoil.

North Carolina.—No. 58. In a sample of sandy loam from a pocosin near Trenton, N. C., no dihydroxystearic acid was isolated. The soil is not cultivated, and although in timber, is said to be a poor soil; the land is not well drained.

No. 59. A peaty soil from the region bordering Lake Mattamuskeet, N. C., did not show the presence of dihydroxystearic acid.

No. 60. From a sample of Norfolk sandy loam from Laurinburg, N. C., said to be a good soil and having good drainage, no dihydroxystearic acid was isolated.

Nos. 61–62. Samples of surface soil and subsoil of Norfolk coarse sandy loam from near Rockingham, N. C., did not reveal the presence of dihydroxystearic acid. This soil is reported to be highly improved and productive. The drainage is excellent.

Nos. 63–64. Samples of surface and subsoil of Norfolk coarse sandy loam from Rockingham, N. C., collected in a field adjoining samples 61 and 62, likewise did not show the presence of dihydroxystearic acid. This soil is not considered as productive as soil No. 61, but is said to be poorly farmed by tenants.

No. 65. Surface soil of Durham sandy loam from Clayton, N. C., showed the presence of dihydroxystearic acid. This soil is from a very poor cotton field, yielding one-fourth to one-third of a bale per acre. Corn has also been planted in the past, as well as other crops, but only very low yields have ever been secured. No barnyard manure has been added in recent years and very little has been used.

No. 66. A sample of Cecil sandy loam from the vicinity of Charlotte, N. C., did not show the presence of dihydroxystearic acid. The field from which the sample was obtained has been cultivated for a very long time and has not been fertilized in recent years and gives very poor yields.

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[1] A Preliminary Report on the Volusia Soils, Their Problems and Management, by M. Earl Carr. Bureau of Soils, U. S. Dept. Agr., 1909.

North Dakota.—No. 67. From Fargo, N. Dak., a sample of Marshall loam was found to contain no dihydroxystearic acid. This is an old sample of soil which has been in the laboratory for several years, and the result is therefore not conclusive as to field conditions.

Oregon. No. 68. A sample of marsh peat from Klamath Marsh, Oreg., contained dihydroxystearic acid.

Pennsylvania.—No. 69. A sample of Hagerstown silt loam from State College, Pa., was found to contain no dihydroxystearic acid. The field from which this sample was taken is in good condition and has good drainage.

No. 70–71. From Bedford County, Pa., samples of Frankstown stony loam, both surface and subsoil, were examined for dihydroxystearic acid. The surface soil was found to contain it, but none could be found in the sample of subsoil. The field from which this soil was taken is very low in productivity and has received no fertilizer of any kind or any lime.

No. 72. A sample of Chester silt loam from near Philadelphia, Pa., was found to contain dihydroxystearic acid. The samples are from portions of a field where crops always failed or were very poor. This sample is from ground in a high state of cultivation, being well manured and cultivated, but is from a spot in which seeds will germinate, but the plants will die or not come to maturity. For instance, celery was planted in this part of the ground after giving it a heavy application of stable manure, but the plants either died or did not grow to any size, whereas on the adjoining ground the plants grew to be at least 2 feet high. Mr. H. J. Wilder, who made a field inspection of this ground, says: "This soil has been well farmed and so far as the field conditions ordinarily considered are concerned this soil should produce excellent yields. The physical condition is good, the organic content is sufficient, drainage is well established, and there is no impervious layer or stratum in the subsoil. Field conditions indicate that more lime should be applied, as horse sorrel is found; but this probable need of lime is not sufficient to account for the spots where various crops are unable to live, or at least to thrive. Hence it seems that there is here a definite case of toxic conditions of some kind."

Nos. 73–74. Samples of Westmoreland silt loam, surface and subsoil, from Clayville, Pa., did not contain dihydroxystearic acid. This is an average soil for this type; no commercial fertilizer or manure has been added in the last six years.

Tennessee.—No. 75. A sample of Clarksville silt loam from the vicinity of Pulaski, Tenn., was found to contain dihydroxystearic acid. This soil was sent in by Mr. Ayrs of this Bureau. "It has been in cultivation for 15 years, for the most part in cotton, and the yields have been light. The surface soil is a gray silt loam 6 inches deep. The subsoil to a depth of 3 feet is yellow silt loam, having a close, compact structure. The field is in a large area of this type, the Clarksville silt loam occupying the broad, nearly level, top of a ridge. There were a few apple trees in the field, but the sample was taken at some distance from any of them. The yields have been light. On an area about 50 feet square, from which the sample was taken, the plants were only about one-half to one-fourth as large as in other parts of the field, and matured very few bolls. Where vacancies occurred in the cotton rows, corn had been planted. The same difference existed in the corn as in the cotton. Near where the sample was taken the stalks were small, with a yellow color, and had failed to form good ears. In other parts of the field the corn had a good color and average-sized ears had been produced. The east half of the field contains 20 apple trees, set five years ago. One tree stands about 30 feet from where the sample was taken and is smaller than the trees farther away, thus agreeing with the growth of the cotton and the corn in different parts of the field."

Texas.—Nos. 76–77. Samples of Amarillo silt loam, surface and subsoil, from Sherman County, Tex., show no dihydroxystearic acid. This is considered a fair soil.

Nos. 78–79. Orangeburg fine sandy loam from Marshall, Tex., surface and subsoil samples, from a good field, one which had received heavy applications of stable manure in connection with the growing of truck crops, showed small amounts of dihydroxystearic acid.

Nos. 80–81. Samples of soil and subsoil of Orangeburg fine sandy loam from the same locality, from a field which had not been fertilized, but which had grown cotton for years and had been allowed to go into native vegetation again, showed much larger quantities of dihydroxystearic acid than the previous sample.

No. 82. Baldwin clay loam from Medina County, Tex., contains dihydroxystearic acid. This is a virgin soil, uncleared.

West Virginia.—Nos. 83–84. Surface and subsoil samples of Dekalb silt loam from the vicinity of Ravenwood, W. Va., were found to contain no dihydroxystearic acid. The field from which these samples were collected has good drainage; the soil is said to be rather unproductive, but to respond rather well to fertilizers and manure. The spot from which the sample was taken was the poorest in the field and one which had received no manure.

OCCURRENCE OF DIHYDROXYSTEARIC ACID IN MANY SOILS.

In the foregoing list a total of 84 soil samples were examined for dihydroxystearic acid; of these 24 were subsoils. Of the total number of samples examined, 27 contained this compound as a constituent part.

The list comprises a total of 60 soils of widely different characteristics, collected in 18 States. They vary from extremely unproductive soils to soils of the highest productivity and represent soils of different origin, different systems of cropping, widely different climatic conditions, and vary in texture from loose sands to plastic clays. Of these soils 33 per cent showed the presence of dihydroxystearic acid. It was contained in soils under long cultivation, as well as in virgin lands; in soils continually cropped, as well as in soils under permanent sod. This shows that this compound is a common soil constituent and likely to be encountered in soil work anywhere. It was found in soils as widely separated as Massachusetts, Texas, Oregon. This at least indicates that there is some force at work which forms this compound in soils everywhere. Its formation or its accumulation is doubtless due to local soil conditions in any one section, but these local soil conditions are not confined to any region of the United States and probably not to any country or continent.

The 60 soils examined may be divided into two classes: (1) Good soils, comprising those of average or high productivity for the soil types represented; (2) poor soils, comprising those less productive than Class 1, as well as those of undoubted infertility.

IN GOOD SOILS.

In the above list 25 of the soils can be classed as good soils; that is, soils of high or average productivity. Of these, only two contained dihydroxystearic acid, both in soil and subsoil. These were the samples of the Dunkirk clay from New York and the Orangeburg fine sandy loam from Texas. Of the former soil type there was no poorer sample sent in, so that no direct comparison of this kind is possible. It is, however, a soil of only fair productivity and the sample may, therefore, not have been so typical of the best fields in this type as should be the case for this classification. It was, however, our judgment, based on the collector's report, that this sample be classed among the good, rather than among the poor soils. With the Texas soil the case is different. Here there is available for comparison a soil sample from an adjoining poorer field of the same type. This poorer field which had not been fertilized and had been so poorly farmed that it was abandoned to native vegetation, contained considerably more than the sample from the better field which had been well manured and well farmed by the growing of truck crops. This is exactly what would be expected in the case of a soil containing this substance when it is improved by manures, fertilizers, and thorough cultivation. Ultimately the dihydroxystearic acid should disappear entirely under such conditions.

IN POOR SOILS.

The soils which can be classified as poor are 35 in number and comprise those less productive than those considered above, as well as some distinctly infertile soils. Of these 35 poor soils, 51 per cent contained dihydroxystearic acid.

Ten of the soils had distinct problems of infertility. These are the Volusia silt loam from New York, the 3 soils from Boston Common, the 2 soils from Redding, Conn., the Elkton silt loam and the Takoma lawn soil from Maryland, the Chester silt loam from Pennsylvania, and the Clarksville silt loam from Tennessee. In everyone of these samples of distinctly infertile soils dihydroxystearic acid was found in appreciable amounts, except in the surface soil of the Volusia silt loam, but here also it was found in the subsoil.

Deducting these 10 very infertile soils, all of which contained dihydroxystearic acid, from the above 35 poor soils, there remain 25 poor soils about which nothing definite is known excepting that they are relatively less productive than other soils in the same regions. Nevertheless, 32 per cent of these contained this soil constituent.

Judging from the foregoing relationships established by this investigation it would seem that dihydroxystearic acid is either a direct or indirect factor in the low productivity in soils; direct by virtue of its harmful effect on growing crops, indirect as an indicator of other compounds or conditions which cause soils to become less productive and even infertile. It is not possible to state from the data at hand that dihydroxystearic acid is the only factor which contributes to the infertility or unproductivity in those soils in which it was found for it must be remembered that this is only one of many compounds, both organic and inorganic, harmful and beneficial, which exist in soils, any and all of which play a part in its relative fertility or infertility. It is certain, however, that the determination of even this one constituent leads to a recognition of the kind of infertility in the soils examined. The poor soils in which it was not found may, for instance, be poor because of other compounds of a harmful nature, although they may also be poor because of the absence of beneficial substances necessary for proper plant development.

IN SUBSOILS.

Wherever dihydroxystearic acid was found in the surface soil it was also found in the subsoil except in two cases, the Frankstown stony loam from Pennsylvania and the Clarksville silt loam from Kentucky.

On the other hand, the sample of Volusia silt loam from New York, as already mentioned, contained it in the subsoil and not in the surface soil. Of the subsoils examined, 24 in number, one-third contained dihydroxystearic acid. The data, therefore, does not permit us to draw any conclusion as to whether it is formed in the surface soil or in the subsoil.

SUMMARY.

This investigation is a partial survey of the nature of soil organic matter in the soils of the United States. Soils from 18 different States, extending from Maine to Oregon and southward to Texas, of widely different origin, topography, texture, climate, drainage, and cropping, varying from soils of the highest productivity to soils incapable of producing profitable crops, were examined for definite organic soil constituents.

To facilitate this examination, the numerous individual methods for the separation of these compounds have been so coordinated as to form a single comprehensive procedure of analysis.

In the soils so examined, pentosans, pentose sugars, histidine, cytosine, xanthine, hypoxanthine, and dihydroxystearic acid were quite frequently encountered and the results indicate that these substances will be found to be widely distributed.

Arginine, agroceric acid, lignoceric acid, α-monohydroxystearic acid, agrosterol, phytosterol, and hentriacontane were only occasionally found, but no general statement as to their limited occurrence can be made.

The frequent occurrence of dihydroxystearic acid is of special interest and significance because of its known harmful properties to plants. One-third of all the soils examined showed the presence of this compound. It was found in virgin soils as well as in soils under long cultivation; in soils continually cropped as well as in soils under permanent sod; in soils from the Atlantic coast; in soils from the Pacific

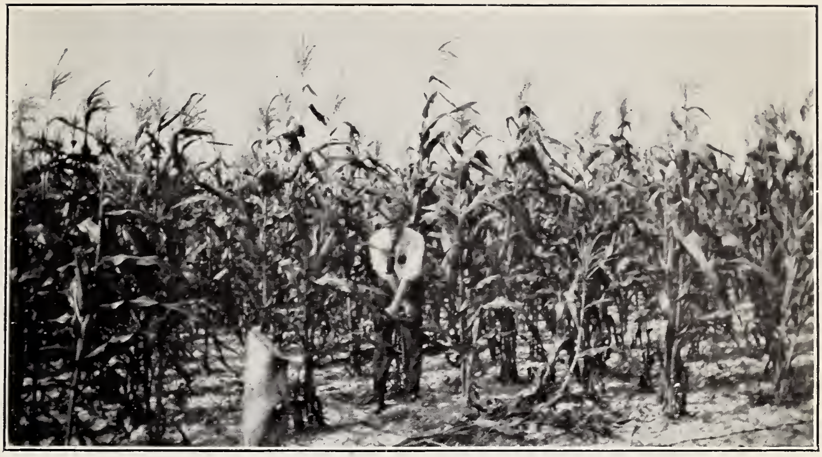

FIG. 1.—GROWTH OF CORN ON SASSAFRAS SILT LOAM, EASTON, MD.

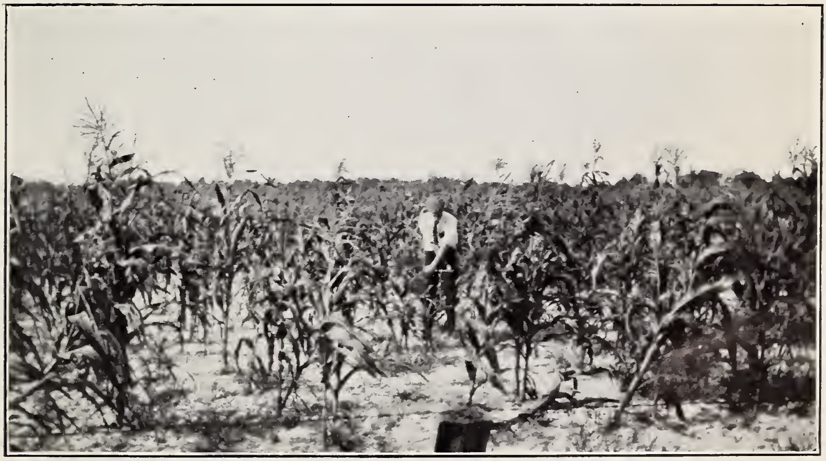

FIG. 2.—GROWTH OF CORN ON ELKTON SILT LOAM 200 YARDS DISTANT FROM THE POINT WHERE FIG. 1 WAS TAKEN.

coast; and in soils from the Gulf States. This compound is, therefore, a common soil constituent and is likely to be encountered in soils anywhere. Its formation or its accumulation is doubtless due to local conditions in any one section, but these local soil conditions are not confined to any region of the United States and probably not to any country or continent.

When the soils examined are separated into good and poor soils, as based on field observations, their relationship with dihydroxystearic acid is rather striking. Among the good soils only two contained dihydroxystearic acid and they were of only moderate productivity. Among the poor soils the percentage of those containing this compound was 51. Of the soils which had a record for infertility, the dihydroxystearic acid was found in each and every case.

Judging from the foregoing relationships established by this investigation it would seem that dihydroxystearic acid is either a direct or indirect factor in the low productivity in soils; direct by virtue of its harmful effects on growing crops, indirect as an indicator of other compounds or conditions which cause soil to become less productive and even infertile. It is not possible to state from the data at hand that dihydroxystearic acid is the only factor which contributes to the infertility or unproductivity in those soils in which it was found, for it must be remembered that this is only one of many compounds, both organic and inorganic, harmful and beneficial, which exist in soils, any and all of which play a part in its relative fertility and infertility. It is certain, however, that the determination of even this one constituent leads to a recognition of the kind of infertility in the soils examined and is, therefore, a readily recognized symptomatic factor of poor soil conditions.

Urban

Barker